J. Animal and Poultry Prod., Mansoura Univ., Vol.5 (4): 221 - 235, 2014

APPLICATION OF *Pichia anomala* And *Schwanniomyces occidentalis,*as A Boicontrol Of Mycotoxigenic *Aspergillus flavus* And Their Effect On The Metabolism Of Rats.

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ABSTRACT

The contamination of animal feed with mycotoxigenic fungi is a disastrous problem along the food chain. *Pichia anomala* and *Schwanniomyces occidentalis* were applied as a biocontrol yeast to prevent *Aspergillus flavus* growth *in vitro* and in feed corn. The yeast strainswere able to inhibit mycotoxigenic fungi growth on animal feeds and improve the properties of the feed.The enzymes assay indicated that *P. anomala* was able to produce β-1,3-glucanase and chitinase which could propose a mode of action for its antifungal activity. Scanning electron micrographs of fungal hypha cultures with *P. anomala* revealed that yeast cell could colonize the fungal hypha leading to their lysis and deterioration. *Schwanniomyces occidentalis*produces enzymes, including a-amylase and glucoamylase,and excretes very efficiently large protein (glucoamylase).The experimental treatments T with rats divided as follows: T1 was fed as basal diet (control), T2 the contaminated diet with aflatoxins (AF) at3ml suspension of fungi/1kg diet from T3:T8wheredifferent concentrations of yeasts were added.T9was similarto T4 without AF and T10 was similar to T6 without AF. The results showed that T2caused significantly lower final body weight and total body weight gain and significantly higher mortality rate, kidney functions(creatinineand urea) and liverfunctions (AST, ALT and ALP) ,beside histopathologicaleffects in Liver, Kidney and spleenthat,Vaculation ofepithelial lining renal tubules, hydropic degemenration of hepatocytes and hemorrhage.On the other hand, the treatmentsT3-T8had significantly an improvement in performance as compared with T2 whichshowedsignificantly higherfinally body weight $p< 0.02\%$ and total body weight gain and lower mortality rate, Kidneyfunctions(CREATININE and Urea) and liverfunctions (AST, ALT and ALP),histopathological examination of in Liver tissue shows activation of Kupffer cells and nohistopathological changes in Kidney and spleen. The results showed the ability of one of yeasts(*P. anomala* or *Schwan.occidentalis*)or theirmixture for inhibiting mold growth andit's toxigenic effects.

Keywords: Yeast killer Fungi, Biocontrol, *Pichia anomala*,*Schwanniomyces occidentalis, Aspergillus flavus*,rat's membranes.

INTRODUCTION

Aflatoxins are the most well-known class of mycotoxins, which are mainly produced by the fungus *Aspergillus flavus*. Aflatoxins are the most potent chemical liver carcinogens known. Moreover, the combination of aflatoxin with hepatitis B and C, which is prevalent in Asia and sub-Saharan Africa, is synergistic, increasing more than tenfold the risk of liver cancer compared with either exposure. Aflatoxins are also associated with stunting in children and possibly immune system disorders (Miller,1994).Aflatoxins contaminated feed stuffs are a potential risk for the consumer because of

their residues in meat- and milk-products.They may contaminate dairy products by molds growing on them, or by the carry-over of mycotoxins occurring in animal feedstuffs ingested by dairy cattle, aflatoxin M_1 is the hydroxylated metabolite of aflatoxin B_1 that may be found in milk or milk products obtained from livestock that have ingested contaminated feed (Van Egmond, 1983).

Acute toxic syndromes and even fatal poisoning of unknown etiology have been observed in beef, dairy and sheep that consumed molded silage (Le Bars and Le Bars 1989 andDriehuis., 2008). *Aspergilli* are the most common fungal species that can produce mycotoxins in food and feedstuffs (Reddy *et al.,* 2009). *A. flavus* is the main producer of aflatoxins B1 and B2which may be metabolized to aflatoxinsM1 and M2 in ruminant milk or milk products after ingestion of contaminated feed.Aflatoxin M1 is cytotoxic, as demonstrated by the results of *invitro* studies in human hepatocytes, and its acute toxicity in several species as similar to that of aflatoxin B1.Contamination of various food and feed stuffs and agricultural commodities with mycotoxins is a major problem especially in the tropics and sub-tropics, where climatic conditions and agricultural and storage practices are favorable for fungal growth and toxin production (Fung and Clark, 2004).

Aflatoxin contamination in corn is a chronic problem across the world that limits maize marketability and causes economic losses. The majority of these sources of resistance lack agronomic performance, which precludes their direct use in commercial hybrids. In addition, no competitive commercial hybrids are available that are resistant to aflatoxin (Chelkowski, 1991 **and Desjardins and Hohn, 1997**).

Since the prevention is always better than cure, so the aim of this study is to prevent the mycotoxigenic *A. flavus* growth in animal feed and consequently secretion of its aflatoxins.

Fleet(1992) and Fredlund*et al*.(2002). .The yeast *Pichia anomala* has been shown to inhibit mold growth in airtight storage systems *Pichia anomala* is among the naturally occurring yeast on grain.

MATERIALS AND METHODS

The microbiologicalresearch in this studywas done in Genetic Engineering and Biotechnology Research Institute(GEBRI) Sadat city, whereas analyses for protein, moisture ,total aflatoxins were done in The Regional Center for Food and Feed - Agricultural Research Center – Giza-Egypt.The Experiment was conducted in the Biological Animal House of the National research Center in Dokki, Giza.The statistical analysis wasconducted at the Research Institute of statistical analysis agricultural Research Center Giza.

Microorganisms

The fungi strain in this study*A.flavus* was obtained from in Genetic Engineering and Biotechnology Research Institute(GEBRI) Sadat city. The yeast strains used in this study,*Pichia anomala* ATCC j121 and *Schwanniomyces occidentalis* ATCC 2322, were maintained in Yeast Malt Extract Broth (YMB).*Schwanniomyces occidentalis* was obtained

fromMcrobiological Resources Centre (MERCIN-CAIRO). *Pichia anomala* was obtained from "Genetic Engineering and Biotechnology Research Institute(GEBRI) Sadat city.

Media:

Yeast Malt extract Broth (YMB):

Consists of 3gyeast extract,3gmalt extract ,5gpeptone and10gglucose.

Potato Dextrose (PDA) Fungi were isolated using media (PDA) suspensionwhich consists of3g yeast extract,3gpotatoes extract, 5gpeptone and10gglucose**.**

PDA suspension was diluted to 10^{-3} with sterile 0.9% NaCl solution and 100 µLof the diluted solution was spread onto a PDA plates. Plates were incubated for 7 days at 25 °C. During this period plates were examined and individual fungal colonies were transferred at first observation to PDA slants.

***Invitro* yeast- fungi direct interaction**

The interaction of yeast with the fungal pathogen hyphae was assessed in Petri dishes containing yeast malt extract agar media (YMB). On the surface of agar, *A. flavus* was inoculated as a single streak in the middle of the plate, and then the yeast cells were inoculated as two spots at the margin of the plates and incubated for 3 days at 28 °C until the growth take place.

Mode of action of the antagonistic yeast*P. Anomala* enzymeassay

(Wang, 1999and Santos and Marquina, 2004)*P. anomala* was cultured in (YMB) with glucose as the sole carbon source. A 250 ml flask containing 100ml culture media was incubated on a rotary shaker at 200 rpm at 28°C for 3 days. Culture filtrate was harvested by centrifuging at 6000 x g for 5 min, and the supernatant was used for enzyme assays.β-1,3-glucanase activity was assayed by measuring the ability of *P. anomala*secretions to degrade β-1,3-glucan. A reaction mixture was prepared by adding 1g of β-1,3-glucan (Sigma) + 2g of agar in 100ml distilled water and shacked well until completely melting, then media were poured in Petri dishes and left to solidify.

After solidification, pores were made on each plate by cork poorer and inoculated each pore by stable amount of filtrate and left for 20-30 min.After that,it was stained with Congo-red (1%) (Which stain the polysaccharides with red color and does not react with monosaccharide) and left for 30 sec.then washed thoroughly with a solution of NaCl (15g of NaCl+500ml distilled water) until developing clear zone. For the chitinase assay, a reaction mixture was prepared by the same method of β -1,3-glucanase assay, but instead of glucan, chitin was used.(knowing that chitin is not soluble in water even after boiling).

Scanning Electron Microscopy(Santosand Marquina,2004). Method was carried out to further observe the direct interaction between fungi and yeast. Also antagonism was carried out in YMB by inoculating the broth firstly with *P. anomala* and then *A. flavus* wasinoculated. After shaking and incubation for 24 h at 28°C, a touch was taken from the broth on magnetic slides and coated with gold-palladium for cell interaction assays.After that, samples were dehydrated in a graded ethanol series, critical-point dried with CO_2 and coated with gold-palladium for cell interaction assays. The tissues were then viewed using a Hitachi S-800 SEM, Japan.

Determination of protein content in yeast supplemented feed

To evaluate the efficiency of biocontrol yeast as feed additives to increase the protein content in corn based feed, 3 days old *P. anomala* cultures in YMB were added at rates of 2, 3 and 4 mg / g of experimentally contaminated corn grain with *A. flavus*. The total protein, calculated from amine nitrogen percentage, in corn grains, either in control group or after addition of yeast culture, was determined using Kjeldahlprotocolaccording to **AOAC (2006)**.

***In vivo* study on rats**

Rats were individually housed in stainless steel metabolism cages for the duration of the trial. The cage floors were made of wire. Feed and water were provided in porcelain containers and glass bottles, respectively. Urine samples were centrifuged at (3640 x *g*) and 1 ml aliquots of the supernatant was placed into 20 ml glass scintillation vials with 100 µl glacial acetic acid and 10ml of scintillate. The samples were left to adapt to the dark for 24 hours prior to analysis by scintillation counting(Walters, 2012).

1- A balanced diet (basal diet),as shown in Table 1.Was offered to rats

Table 1:compositionof the basal diet.

Dietary constituents	Amount	Amount in 14 g
Casein	15.0	2.100
DL methionine	0.3	0.042
Vitamin mixture	1.0	0.140
Mineral mixture	3.5	0.490
Alphacell (cellulose)	3.0	0.420
Corn oil	10.0	1.400
Cholesterol	0.2	0.028
Choline bitartrate	0.2	0.028
Corn starch	66.8	9.352
TOTAL	100	14

2 - Proteincontent in diet with *Pichia anomala* and *Schwanoccidentalis.*In different treatments are presented in Table 2 and the composition of the tested rations are presented in Table 5.

Table 2: Protein content of different treatments.

Treatment	Protein %
T1	7.11± 0.17
T2	7.11± 0.17
T3	8.50± 020
T4	8.95± 0.21
T5	8.30± 0.23
T6	8.93± 0.21
T7	8.47± 0.22
T8	9.25± 0.21
T9	9.81±0.20
T10	10.81±0.21

3–Suspension of fungi and know aflatoxin concentrations in thesesuspension.A balanced diet which moisture is13%. Basal diet according to,Marcia.(2002).Total aflatoxin B1,B2,G1and G2,detection by HPLC according toMansfield,*et. al*. (2005).concentrations in suspension of fungi (s. f) every 1ml (s.f) be given concentration 24ppb.

4 – Preparing media of each yeasts.

5–Fifty rats,albino male and female (*Rattusrattus*).(80:90g) were dividedintoten treatments,five rats in each treatment,T1 was fed a basal diet (control),T2 the contaminated diet with AF.T3-T8,were feddifferent concentrations of yeasts.T9was similar to T4 without AF and T10 was similar toT6 without AF as shown from Table 3.The age of rate in this experiment was 6-7weeks.Each needs approximately 10-15g diet.The duration of the experiment was two months. The temperature was set at 27-30°Cthroughout the experiment.

6 –Collection of blood samples:

Analysis of Blood sample

6. 1 -AST (Aspartate aminotransferase).

6. 2 - ALT (Alanine aminotransferase).

6. 3- ALP (Alkaline phosphatase).according to David and .Karcher(2008).

6. 4- Creatinine.according toSwamy (2002).

6.5–Urea (Urea/Bun-color (Urease/Salicylate).according toJohn and Kratz(2006).

Blood samples were collected from orbital plexus of rat′s eye by fine heparinized capillary glass tubes**,**blood samples wereanalyzedfor liverfunction and kidney function.

- Liver function including: alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (GPT).
- Kidney Functionincluding:creatinine and urea/bun-Color (urease).

7 - Hestopathological techniques: was made on samples of kidney, liver and spleen which were preserved in neutral formalin solution (10%v/v)(North, 1984).

8 – Statisticallyanalysis of the data: statistical analysis was carried out using analysis of variance and least significant difference for testing the significant between various treatments(SAS,2004).Mean values were compared using the Duncan′s New Multiple Range test (Duncan,1955), when significant differences were existed.Significant level was taken at $P < 0.05$.

Table 3:Contentsof the tested ratios.

Treatments	Contents/ 1kg diet
T1	Corn without yeasts or toxin.
T2	Corn + *** toxin.
T3	Corn +toxin+ 2ml y1*.
T4	Corn + toxin+ 4 ml y1.
T5	Corn + toxin+ 2 ml y2**.
T6	Corn + toxin +4 ml y2.
T7	Corn + toxin+ (1 ml y1+ 1 ml y2).
T8	Corn + toxin+ (2 ml y1+ 2 ml y2).
T9	Corn+4 ml y1.
T10	Corn+4 ml y2.

(y1)*P. anomala** ,** (y2)*Schw. occidentalis*,toxin (product of *Asp.flavus*).

RESULTS AND DISCUSSION

In vitro study

Examination of the slide culture under light microscope showed a typical l conidial heads and the shape of colony (Fig. 1) On the PDA medium. a colorless mycelium was observed at the periphery of the colony and a green central part of the colony which referred to the presence of conidia.All these features, in addition to physiological examinations, indicated that the isolated fungus is "*Aspergillus flavus".*

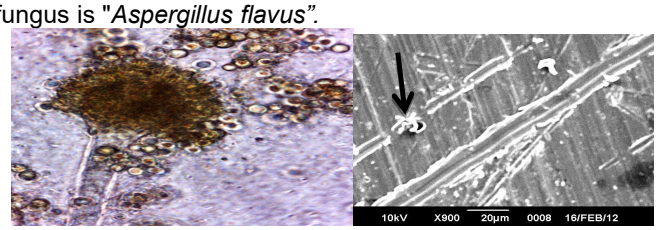


Fig.1.Microscopic features of the isolated *A. flavus*Fig. (2) *P. anomala* and *A. flavus* inmicroscopy.

Fig.1 showing an isolate of *A. flavus* undermicroscopy with high mycelium but Fig.2showing*P. anomala*in antagonismwith*A. flavus* which cutting the mycelium of the fungus.

The antagonism between the boicontrol yeast *P. anomala* and the pathogenic fungus *A. flavus*, on Petri dish containing YMA, revealed that *P. anomala* could effectively prevent the fungal growth as proved by the emptyzone surrounding yeast culture.

P. anomala could produce β-1, 3-glucanase in medium supplemented with β-1,3- glucan, as a sole carbon source. The appeared clear zone increased with incubation time and when the concentration of stain was very low (1%) (Fig .3A). Chitinase activity could be, also, detected for *P. anomala* cultured on medium containing chitin (Fig.3.B).The appeared clear zones could be easily visualized under day light and confirmed the yeast production of both β-1, 3-glucanase and chitinase enzymes.It was reported

that mycoparasitism; destruction or alteration of the hyphae of the pathogen, involving physical contact and predation, followed by enzymatic lysis. Extracellular glucanases and chitinases possess antifungal activity and are involved in mycoparasitism (Castoria*et al.,* 1997andJijakliandLepoivre, 1998).

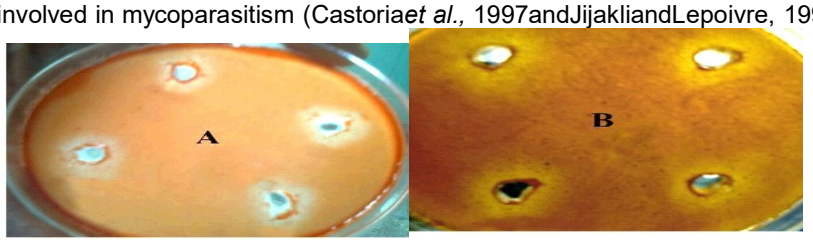


Fig. 3.A: Extracellular enzyme activity in *P. anomala* filtrate measured using Congo red staining.A: β-1,3-glucanase activity ,3.B: Chitinase activity

For further clarification of *P. anomala* mode of action as biocontrol agent against *A. flavus,*The strong hypha colonization produced by *P. anomala* was appeared using an SEM to obtain their attachment in depth and to understand the possible mode of action of this yeast in suppressing the pathogen.The SEM examination showed adherence between hyphe of *A. flavus* and *P. anomala*. In some areas, *A. flavus* hyphae were totally surrounded by the yeast cells (Fig.4B).In other regions, the hyphae of *A. flavus* were totally penetrated and destroyed by the action of the antagonistic yeast (Fig.4A).In captured micrographs, healthy fungal hypha was appeared with normal shape and size (Fig.4a).

***In vevo* study**

Harmonized results were obtained by Chan and Tian(2005), they indicated that *P. anomala* had a stronger capability for attachment to the fungal hyphae of *Moniliniafructicola, Penicilliumexpansum* and *Rhizopusstolonifer* than did *Candida albidus*. On the other hand,the determination of protein content in corn-based feed with *P. anomala,* asprotein supplement, indicated a gradual increase in content from 7.11%, for the unfortified corn, to 9.25%, for the corn fortified with *P. anomala* at supplementation rate of 4mg/kg(Table 2).The calculated percentages of protein increase in the feed were 16.74, 21.65 and 30.10% for the corn feed supplemented with 2, 3 and 4mg/kg, respectively.

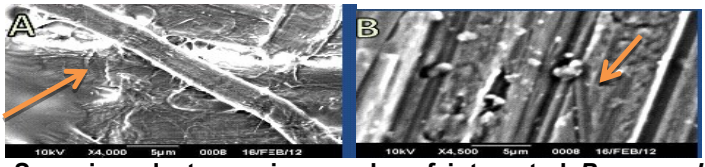


Fig. 4: Scanning electron micrographs of interacted *P. anomala cells* with *A. flavus* after incubation together for 24 h at 28°C.
A: Control *A. flavus* mycelium. B: Cultured fungal mycelia with *P. anomala.*

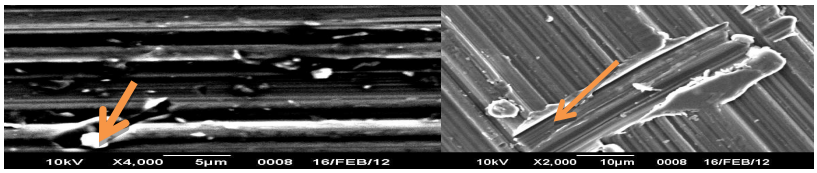


Fig. 5: Scanning electron micrographs of interacted *Schw. occidentaliscells* with *A. flavus* after incubation together for 24 h at 28°C.

The Scanning electron micrographs showed yeast that cutting. A. *flavus* mycelium(Fig. 5).

***In vevo* study**

1- Performance and Mortality.

Table: 4Average body weight of rats.

Treatments	Zero Time	Week1	Week2	Week3	Week4
T1	83.2	114.0^{bc}	117.8^{c}	121.6	101.4^{a}(1M)
T2	85.6	99.6^{e}	99.4^{f}	80.6^{ab} (1M)	60.0^{b} (2M)
T3	82.8	101.8^{de}	110.6^{e}	117.8^{c}	122.6^{a}
T4	83.2	112.0^{c}	113.4^{de}	115.2^{ab}	125.2^{a}
T5	83.6	108.4^{cd}	120.4^{bc}	125.6^{ab}	129.2^{a}
T6	83.4	114.0^{bc}	117.4^{cd}	122.2^{ab}	125.0^{a}
T7	85.4	113.8^{bc}	118.8^{cd}	122.0^{ab}	126.8^{a}
T8	84.6	116.8^{abc}	121.0^{bc}	122.8^{ab}	127.8^{a}
T9	85.6	122.4^{ab}	126.4^{ab}	103.2^{bc} (1M)	105.6^{a}
T10	87.4	124.8^{a}	129.8^{a}	132.8^{a}	138.0^{a}
LSD		8.112	6.339	23.90	40.17
P value	No significance	0.001^{*}	0.001^{*}	0.0065^{**}	0.02^{**}

MMortality,LSD least significant differences. * Significant at 0.05% level - significant at 0.01% level, a high significant, c less significant, bbetween a & c.**

Table (4) shows that, in week 1 T1 (control)reblected an increase in the body weight, T2 had a decrease in body weight as well as 1deth in the third week and 2 deaths in the fourth. In T3-T8 these treatments are (toxin + concentration of yeasts) the effectiveyeasts on toxin increasedthe body weight .The T9 similar toT4 and T10 similar to T6 gave increase in body weight. WhenCompared betweenT7&T10 an increase in body weight was noticed.The present study was in agreement withVelebný*et al.*(2008)who found that,total protein concentration decreased in Groups 1 and 3 with addition of toxin ($P < 0.05$ and 0.01).The results also showed that the toxin contaminated diet significantly decreasedthe performance of rats In comparison with the treatments with theBiocontrol by yeasts.Jansen van Rensburg.(2006)reportedthat 0.5 g of a similar product per kilogram of feed resultedin an increased BW, improved feed conversion ratio, increasedkidney weight, and increased total serum protein and cholesterollevels in broilers at 35 d of age. Serum enzyme activity, hemoglobin,and hematocrit were not affected by the feed additive.

Table (5):Final body weight, body weight gain and mortality rate of testedrats , groups.

Treatments	*FBW	Ranked order	**BWG	***MR%
T1	101.4 (1M) [a]	138.0 [a]	18.2	20%
T2	60.0 (2M) [b]	129.2 [a]	--25.6	60%
T3	122.6 [a]	127.8 [a]	39.8	-
T4	120.2 [a]	126.8 [a]	42	-
T5	129.2 [a]	125.0 [a]	45.6	-
T6	125.0 [a]	122.6 [a]	41.6	-
T7	126.8 [a]	120.2 [a]	41.4	-
T8	127.8[a]	105.6 [a]	43.2	-
T9	105.6[a]	101.4 [a]	20	20%
T10	138.0 [a]	60.0 [b]	50.6	-

FBW Final body weight, ** BWG Body weight gain MR Mortality rate.**
a highersignificant,b lower significant.

Results in Table 5 showed that the body weight of T1had significantly higherfinally body weight (P< 0.0001) than T2 which wascontaminated with the toxin T2: b hadthe lowest significant FBWas compared with other experimental groups and also had the highest mortality rate.The present results arein agreement withthose of He(2013).The third colum showed countdown of FBW. The fourth Colum showed body weight gain (BWG), The decrease in body weight in T2 was due to toxin containing diet which is in agreement withTedesco (2004) andHan.(2008) andkhan. *et,al.* (2010,.who found thatbody weightsafter three weeks of feeding of AFweresignificantlylower than the groups which supplementedwith vitamin E,the AFB1 induced pathological effects in layer breeder hens. In AF fed hens, an ameliorative effect of vitamin E was observed upon AF induced decrease in body weight.

2-Biochemical analysis of blood:

Table 6 showedsignificant decrease in the ratio of toxicity on the liverfunction (AST , ALT and ALP). in T3-T8.

Table6:Average Analysis of blood samplesof different tested treatments.

Treatments	AST ,u/l	ALT u/l	ALPu/l	Creatmg/ml	Urea-N,mg/100ml
T1	70.66^{ab}	27.66^{b}	184.00^{f}	0.700^{a}	51.667^{b}
T 2	75.66^{a}	35.00^{a}	420.33^{a}	0.567^{bc}	63.33^{a}
T 3	63.33^{bc}	27.66^{b}	348.33^{b}	0.567^{bc}	47.66^{bc}
T 4	63.66^{bc}	25.66^{bc}	336.33^{bc}	0.567^{bc}	48.00^{bc}
T 5	62.66^{c}	28.00^{b}	303.33^{cd}	0.500^{c}	42.33^{cd}
T 6	59.33^{c}	23.00^{d}	296.00^{d}	0.500^{c}	38.33^{d}
T 7	60.66^{c}	27.00^{bc}	324.66^{bc}	0.600^{b}	42.33^{cd}
T 8	62.66^{c}	24.33^{cd}	308.33^{cd}	0.533^{bc}	37.33^{d}
T 9	64.33^{bc}	28.33^{b}	223.66^{e}	0.533^{bc}	50.66^{b}
T 10	59.66^{c}	24.33^{cd}	222.33^{a}	0.600^{b}	38.66^{d}
LSD	7.090	2.850	33.89	0.07617	7.636
P value	0.002^{**}	0.001^{*}	0.001^{*}	0.0003^{*}	0.0017^{*}

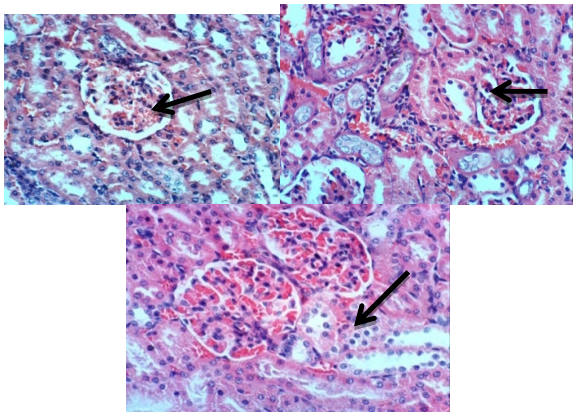
LSDleast significant differences. * Significant at 0.05% level ** significant at 0.01% level. a high significantd less significant .b, care between a & d –P Probabilityvalue

TheKidney function (creatinine and urea) level in rats showed significant alteration when feeding toxin contaminated diet T2since aflatoxin accumulates in tissues throughout the body.Also,the aflatoxinaffectedliverfunctionin T3-T7which showed significant decrease . Microscopically, aflatoxin ingestion hada pronounced dose-response effect on the quantity and severityof hepatic lesions.

.The measurement of alkaline phosphatase (ALP) has long been used in clinical medicine, primarily to aid the diagnosis and monitoring of liver and bone diseases. Its diagnostic utility is limited by the enzyme's wide tissue distribution and by a variety of potential nonpathologic causes. Isoenzyme analysis is one of several tools available to the clinician hoping to further characterize an elevated total ALP. It provides differentiation among liver.David, and Karcher,(2008)

3-Histology

a) Kidney



(Fig.6.a.1)(Fig.6.a.2) (Fig.6.a.3)

Fig.6.a.1 Microhistogram of ratkidneyof control, untreated rat showing the normal histological structure of renal parenchyma(H&E X 400).

Fig.6.a.2 Micro histogram of ratKidney from group (2) showing vacuolation of epithelial lining renal tubules ,a trophy and congestion of glomerular tufts , peritubularinflemnatory cells infiltratinton and thickening of basement membrane of renal tubules. (H % E X 400).

Fig.6.a.3Micro histogram Kidney of rat from group (3) showing congestion of renal blood vessels and thickening of parietal layer of bowman's capsule. (H % E X 400).

Kidney of rat from groups(4,5,6,7,8,9,10) showing no histopathological changes.

b) Liver

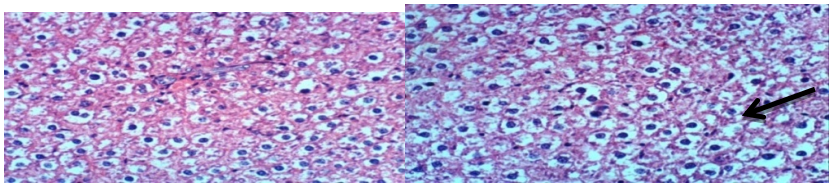
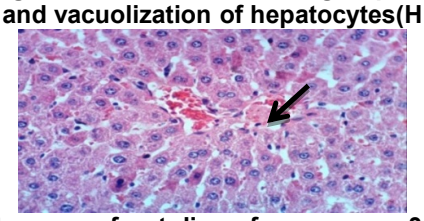


Fig.7.b.1 Fig.7.b.2

Fig.7.b.1 Micro histogram of control liver, untreated rat from group 1 showing the normal histological structure of hepatic lobule.(H & E X 400).

Fig.7.b.2 Micro histogram of ratliverfrom group 2 showing hydropic degemenration and vacuolization of hepatocytes(H & E X 400).



(Fig.7.b.3) Micro histogram of rat liver from group 3 showing necrosis of sporadic hepatocytes and slight Kupffer cells activation.(H & E X 400).

Kupffer cells are found in the liver, which are concerned with the Disposal of old red blood cells, Working on revetment the walls of the cells of the liver and Have a basic relation in the production of gallbladder and called phagocytic cells.

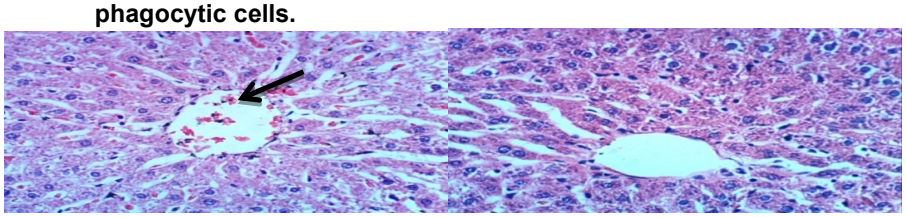


Fig.7.b.4Fig.7.b.5

Fig.7.b.4 Micro histogram of ratliverfrom group (4) showing slight kupffer cells activation and some congestion of hepatic sinusoids. (H & E X 400).

(Fig.7.b.5) of rat liver from group (5,6,7,8,9&10) showing no histopathological changes and kupffer cells activation. (H & E X 400).

c) Spleen

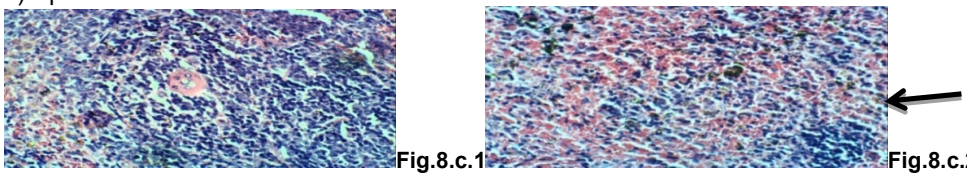
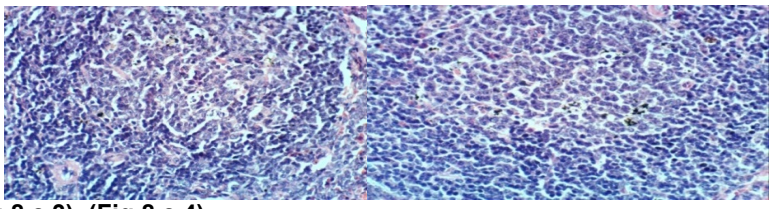


Fig.8.c.1 **Fig.8.c.2**

Fig.8.c.1 Micro histogram ofspleen of control, untreated rat from group (1) showing normal lymphoid follicle (H &E X 400).

Fig.8.c.2 Micro histogram of ratspleenfrom group (2) showing hemorrhage and haemosiderosis (H &E X 400).



(Fig.8.c.3) (Fig.8.c.4)

Fig.8.c.3 Micro histogram of ratspleenfrom group 3 showing slight lymphocytic necrosis
(H &E X 400).

Fig.8.c.4 Micro histogram of ratspleenfrom group (4,5,6,7 ,8,9&10) showing no histopathological changes. (H &E X 400).

Hemorrhage andHaemosiderosis aredisease caused by leaks blood in large quantities leads to laceration many of the body's cells.In the present study, treatment of rats by different doses corresponded and agreed with that done by John and Kratz(2006) .and David., and Karcher(2008),since kidney of T2 showed vacuolation of epithelial lining renal tubules and trophy of glomerular tuft, infiltration of basement membrane of renal tubules but the treatments with doses of yeasts showed no histopathological alterations.Swamy(2002) showed that absolute weights of liver, kidney, and spleen were significantly lower in pigs fed contaminated grains compared with controls ($P < 0.05$). The weights of liver and kidney, expressed as a percentage of body weight, were reduced in the pigs fed contaminated diets ($P < 0.05$), while the relative weight of spleen.The supplementation of 0.05% GM polymer to contaminated diets significantly prevented the decreasedrelative liver weights ($P < 0.05$).

The liver of rats from T2 showed hydropic,degeneration of hepatocytes and vacuolization.When added doses of yeasts began in activating Kupffer cells which disposal of old red blood cells. Working on revetment the walls of the cells of the liver, have a basic relation in the production of gallbladder and called phagocytic cells.Spleen from T2 showed haemorrhage and haemosiderosis and necrosis in slight lymphocytic but when doses of yeasts were added, less begins were recolored than the AF-contaminated diet. These findings agreed with those reported byZahoor(2010).

CONCLUSION

The present results indicated that, aflatoxin is one of the most potent important mycotoxin to rats due to its toxicity. Aflatoxin produces large deleterious and negative effects on rat 's performancemetabolism and organs histology.The results showed that addition of combination of one or both yeasts from 2or 4ml/kg dietto contaminated diet was more efficient in reducing the negative effects on rat.

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تطبيق استخدام الخمائر *Pichia anomala* و *Schwanniomyces occidentalis* كطريقه للمقاومة الحيوية لسموم فطر *Aspergillus flavus* وتأثير ذلك على عمليات التمثيل فى الفئران

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أجرى هذا البحث لتقييم إضافة بعض من نسب الخمائرلمنع وجود الفطريات بالأعلاف حيث أناستمرار تلوثالأعلاف

يعنوجود مشكلة كارثية على طول السلسلة الغذائية. ولقد أستخدمت الخميرتين الحيوانية *Pichia anomala*و*Schwanniomycesoccidentalis* بمثابة المكافحة الحيوية لمنع نمو الفطر (أسبراجلس فلافس) في التجارب المعملية وأيضا على الأعلافوأن تلك الخمائر قادرة على تثبيط الفطر حيث أن الخميرتينتفرزان كلا من الانزيمين كالأتى:

1-*Pichia anomala*(Y1)---------- >β-1، 3-glucanase andchitinase.

2-*Schwanniomycesoccidentalis*(Y2)-->a-amylase and glucoamylase.

وأيضا تعملان على تحسين خصائص الأعلاف،وكما ثبت بالتحاليل أنهما تعملان على زيادة نسبة البروتين بكفاءة كبيرة٠وأ وضح الميكروسكوب الإلكتروني الماسح أن الخميرتين تعملان على تقطيع وتدهور لميسليوم الفطر٠و تم تطبيق ذلك على فئران التجارب وكانت المعاملات كالأتى ١- عليقة خالية من التوكسين و الخمائر،٢- عليقة تحتوى على توكسين فقطوالمعاملات من ٣الى المعاملة ٨ عبارة عن (عليقة +توكسين + نسب من الخميرتين)٠ وأجريت المعاملة ٩ (عليقة +خميرة ١) كمقارنة بالمعاملة ٤ (عليقة +خميرة ١+توكسين)وكذلك المعاملة ١٠ (عليقة +خميرة ٢) كمقارنة للمعاملة ٦ (عليقة +خميرة ٢+توكسين)٠

فأظهرت النتائج أن المعاملة ٢ سجلت انخفاضا معنويا فى وزن الجسم النهائى والزيادة الوزنية الاجمالية وأعلى معدل وفيات ومعدلات عالية فى تحاليلالدم لوظائف الكبد والكلى كما وضح ذلك فى فحص الأنسجة الموضحة بصور للكبد والكلى والطحال حيث تسبب التوكسين فى تهتك أنسجة العديد من الأعضاء وتمزق لخلايا وأوعية بالكبد ووجود فجوات كبدية والخلايا الطلائية بالقنوات الكلوية٠ وعلى الجانب الآخرو بالمقارنة بالمعاملات من ٣ الى ٨ والتى سجلت ارتفاعا معنويا فى وزن الجسم النهائى والزيادة الوزنية الاجمالية وأقل معدل وفيات أيضاانخفاض فى معدلات تحاليلالدم لوظائف الكبد والكلى وعدم تغيرات فى أنسجة الأعضاء , و قد أدى تواجد الخمائر بالعليقة الى تنشيط أنسجة (كوبفر) فى خلايا الكبد٠ ويمكن استنتاج أن أضافة واحدة من الخميرتين او الخلط بينهما لخامات الأعلاف يعمل على تحسين القيمة الغذائية للأعلاف وذلك بزيادة البروتين به وأيضا استخدامهما كمكافحة حيوية للفطرفى حالة ما اذا كانت العليقة ملوثة بالفطر مما يؤدى الى زيادة فترة التخزين للأعلاف دون اصابة فطرية٠